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13. ABSTRACT *(Maximum 200 words)*

We used enzyme and surfactant assays to study digestive capability of a variety of benthic invertebrates, to identify surfactant compounds in animal guts and digestive products of digestion. Gut juices from benthic invertebrates were incubated with polluted sediments and we determined the pollutants released during the incubation. Enzyme and surfactant activities vary across both functional and phyletic categories. Polychaetes have higher enzyme activities than echinoderms, and detritivorous polychaetes have higher protease:lipase ratios than herbivorous and carnivorous species A detritivorous fish had a protease:lipase ratio intermediate to detritivorous and carnivorous polychaetes. Intense surfactant activities were found in deposit feeders. The surfactants from *Arenicola marina* consist of branched C9 fatty acid connected via amide bond to an amino acid. Dissolved trace metal concentrations in digestive fluids are extremely high, with several species having ppm levels. Levels of some metals are proportional to amino acid concentrations. Metal enrichments in gut fluids, relative to sediments, showed the Irving-Williams order. Polycyclic aromatic hydrocarbons (PAH) were solubilized by digestive fluids much more than by seawater. PAH solubilization was found to depend strongly on the solid-solution ratio and sedimentary organic carbo content. These two sets of results imply a strong, fugacity-driven partitioning between digestive fluid and sediment.

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OBJECTIVES: (1) Determine digestive physiology of deposit feeders; (2) Determine the fraction of total pollutants in sediments released during incubation of polluted sediments with digestive fluid of deposit feeders; (3) Examine partitioning of pollutants in sediments, mechanism of digestive fluid solubilization, and design an *in vitro* method to measure their bioavailability.

APPROACH: (1) Use enzyme and surfactant assays to study digestive capability of a variety of benthic invertebrate animals for lipid component of sediments; identify surfactant compounds in animal guts and digestive products of lipid digestion; (2) Extract gut juices from benthic invertebrates and incubate them with polluted sediments, followed by measurement of pollutants released during the incubation; (3) After determining patterns of digestive agents in various animals, design cocktail of commercially available enzymes and surfactants that can be used to mimic the pollutant release kinetics found in part (2), and to explore phase associations of sedimentary pollutants.

ACCOMPLISHMENTS: We surveyed the digestive enzyme and surfactant activities of 18 benthic invertebrate animals and a detritivorous freshwater fish common in the lower Great Lakes. We focused on deposit feeders from the U.S. east and west coasts, but included carnivores, herbivores, and filter feeders for context. Enzyme and surfactant activities vary across both functional and phyletic categories. Polychaetes have higher enzyme activities than echinoderms, and detritivorous polychaetes have higher protease:lipase ratios than herbivorous and carnivorous species. A detritivorous fish had a protease:lipase ratio intermediate to detritivorous and carnivorous polychaetes. Intense surfactant activities are found in deposit feeders. Less intense surfactancy is found in carnivores, herbivores and filter feeders. The surfactants from *Arenicola marina* were found to consist of branched C9 fatty acid connected via amide bond to an amino acid, either leucine or glycine. The fatty acids show branching and unsaturation. Feeding experiments with an omnivorous polychaete showed that varying diet could induce changes in protease:lipase ratio similar to cross-phyletic patterns. Presence or absence of sediment in the diet had no effect on the surface tension of digestive fluid, but sediment did induce higher surfactant concentrations, as indicated by micelle formation. Thus diet can affect digestive chemistry that can in turn affect contaminant solubilization. Gut fluids have high dissolved

organic matter concentrations, with some polychaetes showing dissolved amino acids alone of 5-10%. Amino acids make up roughly half of the dissolved organic matter. Lipids are much less concentrated. Circumstantial and experimental work showed that animals can concentrate dissolved materials (e.g., nutritional amino acids, PAH, and radiolabelled polymers) in gut fluids by retaining fluids relative to solids. These enrichments will affect subsequent absorption.

Dissolved trace metal concentrations in digestive fluids are extremely high, with several species having ppm levels. Levels of some metals are proportional to amino acid concentrations. Metal enrichments in gut fluids, relative to sediments, showed the Irving-Williams order, consistent with soft ligands (e.g., amino acids) as the solubilizing agents.

Metals in contaminated sediments were released during incubation with digestive fluid to a much greater extent than in incubation with seawater. Kinetics of release to digestive fluid were complex among metals and sediments, including even biphasic patterns with opposite signs (early adsorption followed by release). For highly contaminated sediments, the release rate for a metal like Cu is slow enough that incomplete solubilization will occur during gut passage of sediment through the animal. These kinetic patterns will affect the animals' ability to bioaccumulate metals.

The amount of Cu solubilized by different molecular weight fractions of digestive fluid is related to their peptide content. Solubilization is due to complexation by proteins rather than their enzymatic activity. We could mimic Cu release with solutions of off-the-shelf proteins at similar concentrations as are found in digestive fluid. Histidine residues are critical for Cu binding by digestive fluids. Both high molecular weight proteins, representing enzymes secreted by the animal, and lower molecular weight peptides, representing nutritional material solubilized from sediment, were important in releasing Cu from contaminated sediments. Low molecular weight fractions were found to be more effective solubilizing agents, per amino acid residue, likely due to greater exposure to solution. Ion-specific electrodes were used to determine conditional binding constants between Cu and gut ligands, and ranged from 10^{-4} to 10^{-14}, consistent with known histidine affinities for Cu.

Comparison of digestive fluid extraction with the acid-volatile sulfide (AVS) method showed agreement. Digestive solubilization of borderline metals (Cu, Cd, Ni, Zn, Pb) occurred only when these metals were present in concentrations in excess of the AVS, consistent with the relative binding affinities of histidine vs. sulfide.

Solubilization of Cu from sediment can result in inactivation of digestive fluid enzymes. Inactivation occurs at similar ratios of Cu to gut amino acids, across enzyme type and animal species.

Polycyclic aromatic hydrocarbons (PAH) were also found to be solubilized by digestive fluids much more than by seawater. PAH solubilization was found to depend strongly on the solid-solution ratio and sedimentary organic carbon content. At in vivo solid-solution ratios, PAH solubilized by digestive fluid was inversely dependent on sedimentary OC concentration. Increasing the solid-solution ratio

decreased the proportion of total PAH extractable by digestive fluid. These two sets of results imply a strong, fugacity-driven partitioning between digestive fluid and sediment. Using dilution experiments that tested for PAH solubility above and below the critical micelle concentration (CMC) of the surfactants in gut fluids, we established an important role of surfactants in PAH solubilization. Solubilities of heavy and light PAH are similar in gut fluid micelles, presumably due to similar limitation by micellar volume. Proteins may also solubilize PAH and other hydrophobic materials (e.g., methyl Hg). PAH solubilization from contaminated sediments is much less than from pure PAH solids, implicating competition for micellar space from other sedimentary lipids. PAH solubilization from highly contaminated sediment is also limited by saturation of the micelles. Hence bioavailability from such sediments will be more a function of surfactant secretion by the animal than of inherent solubility from the sediment matrix. Repeatedly incubating sediment with fresh batches of gut fluid released similar amounts of PAH, consistent with saturation behavior. The kinetics of PAH solubilization are rapid, reaching completion within a gut residence time. Absorption of PAH by gut walls is dominantly by passive diffusion.

CONCLUSIONS: Digestive fluid contains agents - e.g., amino acids and surfactants - that significantly increase exposure of benthic animals to contaminants during digestion. Variations in bio/chemical properties among animal species and among sediments can explain patterns of bioavailability among animal-sediment combinations. The interactive chemistry of these two "reactants" governs bioavailability.

SIGNIFICANCE: Lack of understanding of bioavailability is the major hindrance in the application of science to contaminant management issues. This study provides a scientific basis for the failure of current regulatory models to explain animal exposure to sedimentary contaminants in harbors. It provides a basis for more accurate understanding and routine determination of contaminant bioavailability and hence risk assessment.

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